Online Supplementary Material

Maternal iron kinetics and maternal-fetal iron transfer in normal-weight and overweight pregnancy

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Preparation of isotopically labeled iron

Isotopic labeled ^{57}Fe- and ^{58}Fe-ferrous sulfate was prepared from isotopically enriched elemental iron by dissolution in diluted sulfuric acid [1]. The solutions were stored in perfluoroalkoxy alkane (PFA) containers and flushed with argon to keep the iron in the +2 oxidation state.

Calculation of fractional iron absorption in pregnancy

Accurate estimates of blood volume are critical for calculations of iron absorption using stable isotope techniques. We used plasma expansions of 4.4% at pregnancy week 12, 19% at week 20, 25% at week 22 and 45% at weeks 30, 34 and 36 [2]. Our estimates (Table 1) in the 1st trimester are comparable to measured blood volume in non-pregnant women [3] and our estimates of blood volume in the 3rd trimester are comparable to measured blood volume in lean (95 ± 30 ml/kg BW) and obese (73 ± 22 ml/kg BW) pregnant women in the third trimester [4]. Our estimates of plasma volume and red cell volume expansion are also consistent with most previous studies in pregnant women supplemented with iron that reported increases in plasma volume of ≈50% and in red blood cell mass of ≈35% [5, 6]. We excluded iron absorption data from the first test meal in one NW woman and from the second test meal in one NW woman and one OW woman from the data analyses because of an error in isotope administration. We calculated fractional iron absorption (FIA) assuming an 80% incorporation of absorbed iron into maternal erythrocytes [7], but previous studies measuring incorporation during pregnancy have produced varying results. In a previous study in 12 pregnant women (23–36 wk gestation), erythrocyte incorporation of an intravenous tracer was 64.7 ± 12.2% [8]. In another study, mean (SD) erythrocyte incorporation of an intravenous label was 63.4 (12.1) % in early pregnancy and 71.0 (10.4) % in late pregnancy, significantly lower than in non-pregnant women (90.1 (SD 6.0) %) [9]. Finally, in the study of O'Brien [10], mean ± SD incorporation of an intravenous tracer was lower in the iron-supplemented pregnant women (76.4 ± 13.1) than in the non-supplemented group (91.5 ± 28.0). We based our incorporation value of 80% on the study of O'Brien [10] as the methods of that study most closely matched ours.

Statistical analysis

Data was checked for normal distribution by visual inspection and by Shapiro-Wilk testing. Non-normally distributed data were logarithmically transformed for statistical analyses. Data was expressed as mean ± SD (for normally distributed data) or median (IQR) (for non-normally distributed data). For

between-group effects, independent sample t-tests or nonrelated samples non-parametric tests were used. We used linear mixed effect model analysis (LMM) with post hoc Bonferroni correction to assess the effect of the group (NW vs. OW) and time (PW12, 20, 30, 36) on different variables. Group and time were defined as fixed effects, participants as random intercept effects using a compound symmetry structure matrix. For the LMM on FIA we included SF, TfR and age as covariates. We used linear regression analyses on iron and inflammation status parameters and FIA to assess the effect of prepregnancy BMI and inflammation. We sequentially added known potential predictors to the models. We used Spearman's correlation analysis to determine predictive power of prepregnancy BMI on upregulation of FIA from the second to the third trimester.

Because prevalence of inflammation was low, we did not adjust infant's iron status parameters for inflammation. We used linear mixed effect model analysis (LMM) with post hoc Bonferroni correction to assess the effect of the group (infants born to NW mothers vs infants born to OW mothers) and time (at age 3 days, 3 months, 6 months) on different variables. Group and time were defined as fixed effects, participants as random intercept effects using a variance component structure matrix. For the LMM on SF, sTfR and body iron stores, we included AGP as a covariate. Maternal fetal transfer was determined using the isotopic labels and total circulating iron. The proportion of absorbed first label in the newborn was compared to the sum of absorbed label in the mother at week of pregnancy 22 and the baby at age 3 days. The proportion of absorbed second label in the newborn was compared to the sum of absorbed label in the mother at week of pregnancy 32 and the baby at age 3 days. The proportion of total circulating iron (Fe_{circ}) transferred from the mother to the infant was calculated from the ratio of circulating iron in the infant at age 3 days and circulating iron in the mother at week of pregnancy 36. We used linear regression analyses on portion of label and circulating iron transferred from the mother to the infant and on body iron stores in the infant over the first six months to assess the effect of prepregnancy BMI. We sequentially added known potential predictors to the models. We used Spearman's correlation analysis to determine the accordance between iron and inflammation parameters measured in cord blood an in the newborn at age 3 days. To compare parameters measured in cord blood and in the newborn at age 3 days, we used independent sample t-tests or nonrelated samples non-parametric tests.

We assumed the first tracer, administered in ≈PW 20 to the mothers, had uniformly equilibrated during gestation in the newborn [11]. In a subgroup of infants, we determined the fraction of total body iron absorbed per day (k_{abs}), i.e. the rate of dilution of the first administered tracer, during the first (NW: n=15; OW: n=13) and the second (NW: n=14; OW: n=13) three months of life. We determined k_{abs} as the slope of the natural logarithm of the concentration of the first administered tracer in cord blood samples and blood samples at three and six months of age plotted against age [11]. We only included infants into the analysis on k_{abs} and ΔFe_{circ} where we had a measure of k_{abs} and ΔFe_{circ} for both the 0-3 months and the 3-6 months period (Nw: n=12; OW: n=11). In the independent sample t-tests on k_{abs} and ΔFe_{circ} we did bootstrapping with a resampling size of 1000. We used linear mixed effect model analysis (LMM) to assess the effect of the group (infants born to NW mothers vs infants born to OW mothers) and time (0-3 months, 3-6 months) on k_{abs} and ΔFe_{circ}.

Limitations of interpreting cord blood iron biomarkers

Previous studies [12-18] assessing the effect of OW on newborn iron stores only assessed parameters in cord blood. Cord blood measurements of most iron biomarkers are likely confounded by the physiologic effects of delivery. For example, cord blood hepcidin concentrations are generally higher than maternal concentrations and typically show no correlation with maternal hepcidin at delivery [6]. Unlike previous studies, we measured variables in both cord blood and in the newborn at age 3 days

from n=47 mother-infant pairs. Comparing parameters in cord blood versus age 3 days, although there were significant correlations in many biomarkers: SF (r_s = 0.518, p=0.0002), TfR (r_s = 0.446, p=0.0017), body iron stores (r_s = 0.504, p=0.0003), AGP (r_s = 0.349, p=0.0162) the absolute values varied widely and differed significantly (p<0.05 for all) (Table 3). For example, SF was 35% higher in 3 day blood vs cord blood and CRP concentrations were 8-fold higher in 3 day blood versus cord blood. These findings suggest interpreting hepcidin and iron biomarkers from only cord blood samples as true 'newborn' values may be problematic.

Supplemental Table 1. Maternal characteristics in the postpartum.

	3 months	**6 months**	**p-values**		
			group	**time**	**Group*time**
Time after delivery			NA	NA	NA
NW	3.8 (3.5-4.3) (n=23)	6.5 (6.1-6.9) (n=29)			
OW	3.7 (3.4-4.2) (n=23)	6.7 (6.5-7.5) (n=27)			
Weight, kg			<0.001	0.7010	0.0004
NW	58.6 (53.7-64.8) (n=20)	59.3 (51.4-63.3) (n=28)			
OW	78.4 (69.8-92.8) (n=22)	78.2 (71.7-92.6) (n=27)			
Hemoglobin, g/dl			0.6838	0.3456	0.1568
nw	13.1 (12.5-13.6) (n=23)	13.5 (12.9-14.0) (n=29)			
OW	13.2 (12.6-13.8) (n=23)	13.4 (13.0-14.1) (n=26)			
SF, µg/L			0.9494	0.2025	0.2237
NW	44.8 (26.2-73.0) (n=21)	55.8 (32.4-98.8) (n=27)			
OW	45.1 (26.9-93.0) (n=23)	53.4 (38.7-80.6) (n=25)			
sTfR, g/L			0.0154	0.8187	0.8349
NW	4.4 (3.8-5.5) (n=21)	4.6 (3.9-5.9) (n=27)			
OW	5.4 (4.6-6.7) (n=23)	5.2 (4.7-6.4) (n=25)			
BIS, mg/kg BW			0.4322	0.2138	0.3202
NW	6.8 (4.4-8.9) (n=21)	7.0 (5.8-9.2) (n=27)			
OW	6.7 (4.0-9.2) (n=23)	6.5 (5.7-8.4) (n=25)			
RBP, µmol/L			0.2924	0.3496	0.6587
NW	1.69 (1.17-1.91) (n=21)	1.58 (1.35-2.15) (n=27)			
OW	1.59 (1.50-2.30) (n=23)	1.75 (1.37-2.13) (n=25)			
CRP, mg/L			<0.001	0.4725	0.9191
NW	0.93 (0.55-2.63) (n=21)	0.94 (0.50-1.94) (n=27)			
OW	4.36 (2.69-9.20) (n=23)	3.71 (2.02-9.18) (n=25)			
AGP, g/L			0.0004	0.1149	0.2673
NW	0.63 (0.52-0.86) (n=21)	0.69 (0.46-0.87) (n=27)			
OW	1.01 (0.78-1.21) 1.02 (n=23)	0.89 (0.72-1.08) (n=25)			

Data as median (IQR). Linear mixed-effect model analysis was performed with group and time as fixed effects. AGP: alpha-1-glycoprotein, BIS: body iron stores, BW: body weight, CRP: C-reactive protein, IL-6: interleukin-6, NW: normal-weight, OW: overweight, RBP: retinol-binding protein, SF: serum ferritin, sTfR: soluble transferrin receptor.

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